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Synthesis and evaluation of antitumor, anti-inflammatory and analgesic activity of novel deoxycholic acid derivatives bearing aryl- or hetarylsulfanyl moieties at the C-3 position

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ABSTRACT

Novel deoxycholic acid (DCA) derivatives were stereoselectively synthesised with –OH and –CH_2SR moieties at the C-3 position, where R was a substituted aryl [2-aminophenyl (**8**) or 4-chlorophenyl (**9**)] or hetaryl [1-methylimidazolyl (**5**), 1,2,4-triazolyl (**6**), 5-amino-1,3,4-thiadiazolyl (**7**), pyridinyl (**10**) or pyrimidinyl (**11**)]. These compounds were prepared in good yields from the C-3β-epoxy derivative **2** in the epoxide ring-opening reaction by S-nucleophiles. These derivatives were evaluated for their *in vitro* anti-proliferation activity in a panel of tumor cell lines. Data showed that: (i) heterocycle-containing derivatives displayed higher cytotoxicity profiles than the parent molecule; (ii) heterocyclic substituents were more preferable than aryl moieties for enhancing anti-proliferation activity; (iii) the sensitivity of tumor cell lines to analysed compounds decreased in the following order: HuTu-80 (duodenal carcinoma) > KB-3-1 (cervical carcinoma) > HepG2 (hepatocellular carcinoma) > MH-22a (hepatoma); (iv) compounds **5**, **6** and **11** exhibited a high cytotoxic selectivity index (HuTu-80: SI > 7.7, 38.5 and 12.0, respectively). Compounds **2** and **6–8** markedly inhibited NO synthesis by interferon γ-induced macrophages. Screening for anti-inflammatory activity of these derivatives *in vivo* showed their high potency on histamine- (**5**, **10**) and formalin- (**2**, **10**, **11**) induced paw edema models.

1. Introduction

Bile acids (BAs) are steroid metabolites that are major components of bile. All BAs consist of a rigid steroid nucleus usually containing one to three hydroxyl groups and a short aliphatic side chain with a carboxyl group at the end. BAs are widespread in nature; they have high enantiomeric purity and a broad spectrum of native biological activities (anti-inflammatory, anticancer, cytoprotective, neuroprotective, antifungal, antimicrobial, etc.) [1–5]. Their main functions include solubilisation and transport of lipids and fat-soluble vitamins as well as the regulation of lipid, glucose and cholesterol homeostasis [5–7]. BAs are known to circulate in the human body between the hepatobiliary and intestinal compartments in a process known as enterohepatic circulation [2,8–10]. Structural peculiarities, the reactivity of BAs and their diverse native biological activities makes these scaffolds an interesting and promising starting material for the synthesis of novel compounds with valuable biological activities [5,11].

Current approaches to modify BAs cover a wide range of chemical transformations from the synthesis of derivatives of the carboxylic group (salts, esters, amides) to significant alterations in the steroid skeleton (etherification, substitution, elimination of native hydroxyl groups, introduction of new functional groups). BAs are frequently conjugated to biologically active molecules to enhance their bioavailability and/or reduce toxicity of the latter. Indeed, BA platinum complexes [12,13] and BA conjugates with 3′-azido-3′-deoxythymidine [14] exhibiting anticancer activity have been synthesised. Conjugates of BAs with the antiviral drug acyclovir have also been described [15]. A number of biologically active synthetic derivatives of BAs are known

Abbreviations: BAs, bile acids; DCA, deoxycholic acid; DXR, doxorubicin hydrochloride; IFNγ, interferon γ; i.p., intraperitoneally; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NOS, NO synthases; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; SM, Soloxolone methyl

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[5,11]. For example, introduction of nitrogen-containing functional groups (ammonium, amino or imino groups) into the steroid skeleton generates derivatives exhibiting antimicrobial activity [16–19] and some amides of BAs possess antifungal activity [20–22]. Antitumor activity was found for some ester [23], amide [24,25] and other BA derivatives [26,27], and the receptor modulating properties of BA derivatives have been widely investigated [5]. It is worth mentioning that the biological activity of novel synthetic derivatives of BAs is not always investigated; however, when biological activity is studied it preferentially focusses on their anti-proliferative, anti-fungal, anti-bacterial or anti-microbial activities [5,11].

Recently, we have shown that the introduction of polar functional groups into ring A of deoxycholic acid (DCA) derivatives, such as an isoxazole ring, hydroxymethylene or cyano groups, increases cytotoxicity and anti-inflammatory activity of the compounds relative to DCA and corresponding precursors [28]. Also, it was shown that modification of the steroid skeleton by different heteroatom-containing functional groups, which include heterocyclic moieties, augments and/or creates new valuable biological activities [23–25,29–35]. Heteroatoms may be involved in the formation of additional hydrogen bonds with the receptors, which leads to changes in their biological activity [25,29–31,34,36,37]. The hydrophobic steroid skeleton enables transport through biological membranes, and the presence of the heteroatom-containing moiety may alter the pharmacodynamic and pharmacokinetic parameters of the starting compound [35,38]. It should be noted that the literature provides some examples of sulfur-containing BA derivatives where the steroidal skeleton [39–43] or side chain [36,44–49] has been modified, but the biological activities of these compounds were either investigated poorly or not at all. Nitrogen-containing heterocyclic moieties such as imidazole [50], triazole [51], thiadiazole [52], pyridine, pyrimidine [53] and others are often core structure in many synthetic compounds exhibiting a broad range of biological activities which includes anticancer [50–53], anti-inflammatory [50–52] and analgesic [50] activity. Aromatic ring system also may provide compound with anticancer activity [54]. Therefore, investigation of the biological properties of BA derivatives containing heterocyclic moieties and sulfur-containing groups are of particular interest.

Given these gaps in the literature, the present study was aimed at i) modifying DCA via the introduction of aromatic substituents, including heterocycles, into the steroid skeleton using a ring-opening reaction of epoxy-derivatives; ii) analysing the biological activities of the synthesised compounds including their effect on tumor cell proliferation, NO overproduction and phlogogen-induced inflammation; iii) identifying structure-activity relationships (SARs) based on the series of obtained derivatives.

2. Experimental

2.1. Synthesis

2.1.1. General experimental procedures

Elemental analyses were determined with a EURO EA3000 automated CHNS-analyser. Analyses indicated by the symbols of the elements were within ±0.4% of theoretical values. Melting points were determined on a METTLER TOLEDO FP900 thermosystem and are uncorrected. The elemental composition of the products was determined from high-resolution mass spectra recorded on a DFS (double focusing sector) Thermo Electron Corporation instrument. Optical rotations were measured with a PolAAr 3005 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on Bruker spectrometers: AV-600 (operating frequency 600.30 MHz for ^{1}H and 150.95 MHz for ^{13}C) and DRX-500 (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C). Solutions of each compound were prepared in $CDCl_3$. Chemical shifts were recorded in δ (ppm) using δ 7.24 (^{1}H NMR) and δ 76.90 (^{13}C NMR) of $CHCl_3$ as internal standards. Chemical shift measurements were given in ppm and the coupling constants (J) in hertz (Hz). The structure of the compounds was determined by NMR using standard one-dimensional and two-dimensional procedures (^{1}H–^{1}H COSY, ^{1}H–^{13}C HMBC/HSQC, ^{13}C–^{1}H HETCOR/COLOC). The purity of the final compounds and intermediates for biological testing was >98% as determined by HPLC analysis. HPLC analyses were carried out on a MilichromA-02, using a ProntoSIL 120-5-C18 AQ column (BISCHOFF, 2.0 × 75 mm column, grain size 5.0 lm). The mobile phase was Millipore purified water with 0.1% trifluoroacetic acid at a flow rate of 150 μL/min at 35 °C with UV detection at 210, 220, 240, 260 and 280 nm. A typical run time was 25 min with a linear gradient of 0–100% methanol. Flash column chromatography was performed with silica gel (Merck, 60–200 mesh). All course of all reactions were monitored by TLC analysis using Merck 60 F254 silica gel on aluminum sheets with the eluent $CHCl_3$-MeOH (20:3) or $CHCl_3$-AcOEt (20:3).

2.1.2. Reagents

DCA (99%) was purchased from abcr GmbH & Co. KG. 2-Mercapto-1-methylimidazole, 5-amino-1,3,4-thiadiazole-2-thiol, 4-chlorotiophenol, 2-aminotiophenol, 2-mercaptopyridine, 2-mercaptopyrimidine, trimethylsulfoxonium iodide and trimethylsulfonium iodide were purchased from ACROS organics. Sodium hydride 57–63% in oil dispersion was purchased from Alfa Aesar. 1H-1,2,4-triazole-3-thiol was purchased from Maybridge. All solvents used in the reactions were purified and dried according to previously reported procedures.

2.1.3. Reaction of methyl 3,12-dioxo-5β-cholan-24-oate (1) with dimethylsulfoxonium methylide

Methyl 3,12-dioxo-5β-cholan-24-oate **1** (2.0 g, 5.0 mmol) in DMSO (5.5 mL) was stirred at room temperature until a homogeneous suspension formed. NaH (57–63% in oil, 0.32 g, 7.5 mmol) was stirred in *n*-hexane (12 mL) for 20 min. After decanting the solvent, DMSO (7.5 mL) and trimethylsulfoxonium iodide (1.66 g, 7.5 mmol) were added. The resulting mixture was stirred for 45 min at room temperature (to form the dimethylsulfoxonium methylide). Then a suspension of **1** in DMSO was added to the methylide and the reaction mixture was stirred for 2 h at room temperature. Then the reaction mixture was diluted with distilled water and a white precipitate was filtered, washed with distilled water and dried in air. The crude product **2** (1.94 g, 94%) was a white solid. An analytically pure sample of **2** (0.29 g, 58%) was obtained by flash column chromatography (silica gel, 0.2–4% AcOEt in $CHCl_3$) of crude product (0.5 g) and subsequent re-crystallisation from CH_3OH. Mp 140.7 °C. $[\alpha]_D^{28}$ +128° (*c* 0.10 g/100 mL; $CHCl_3$). HRMS: *m/z* calcd. for $C_{26}H_{40}O_4$: 416.2921; found: 416.2922. Anal. Calcd. for $C_{26}H_{40}O_4$: C, 74.96%; H, 9.68%; O, 15.36%; found C, 75.84%; H, 9.76%; O, 15.23%. ^{1}H NMR ($CDCl_3$): δ = 3.62 (s, 3H, CH3-25), 2.59 (d, 1H, ^{2}J = 4.7, H-26), 2.57 (d, 1H, ^{2}J = 4.7, H-26′), 2.50 (dd, 1H, $^{2}J = J_{11a,9}$ = 12.6, H-11*a*(β)), 2.35 (m, 1H, H-23), 2.26–2.18 (m: 2H, 2.25 (dd, 1H, $^{2}J = J_{4a,5}$ = 13.8, H-4*a*(α)), [2.22]-H-23′), 2.02 (dd, 1H, ^{2}J = 12.6, $J_{11e,9}$ = 4.7, H-11*e*(α)), 1.99 (dd, 1H, $J^{1} = J^{2}$ = 9.0, H-17), 1.94–1.87 (m: 3H, [1.90]-H-2*a*(α), [1.90]-H-6, [1.90]-H-16), 1.87–1.65 (m: 5H, [1.83]-H-8, [1.79]-H-22, [1.78]-H-5, [1.77]-H-9, [1.70]-H-15), 1.59 (ddd, 1H, ^{2}J = 14.4, $J_{1e,2a}$ = 3.8, $J_{1e,2e}$ = 2.7, H-1*e*(α)), 1.50 (m, 1H, H-7*e*(β)), 1.40 (ddd, 1H, $^{2}J = J_{1a,2a}$ = 14.4, $J_{1a,2e}$ = 4.0, H-1*a*(β)), 1.39–1.19 (m: 6H, [1.35]-H-14, [1.34]-H-22′, [1.32]-H-15′, [1.31]-H-16′, [1.26]-H-20, [1.23]-H-6′), 1.06 (m, 1H, H-7*a*(α)), 1.05 (s, 3H, CH_3-18), 1.00 (s, 3H, CH_3-19), 0.94 (dddd, 1H, ^{2}J = 14.1, $J_{2e,1a}$ = 4.0, $J_{2e,1e}$ = 2.7, $J_{2e,4e}$ = 2.5, H-2*e*(β)),0.80 (d, 3H, $J_{20,21}$ = 6.7, CH_3-21), 0.79 (ddd, 1H, ^{2}J = 14.4, $J_{4e,5}$ = 4.5, $J_{4e,2e}$ = 2.5, H-4*e*(β)). ^{13}C NMR ($CDCl_3$): δ = 213.67 (s, C-12), 173.79 (s, C-24), 58.25 (d, C-14), 58.05 (s, C-3), 57.01 (s, C-13), **52.85 (t, C-26)**, 50.80 (q, C-25), 46.06 (d, C-17), 43.25 (d, C-9), 39.79 (d, C-5), 37.88 (t, C-11), 35.11 (d, C-20), 35.04 (d, C-8), 34.92 (s, C-10), 33.65 (t, C-1), 32.95 (t, C-4), 30.74 (t, C-23), 30.07 (t, C-22), 27.40 (t, C-2), 27.03 (t, C-16), 25.97 (t, C-6), 25.42 (t, C-7), 23.83 (t, C-15), 22.36 (q, C-18), 18.10 (q, C-21), 11.18 (q, C-19).